

Northumbria Research Link

Citation: Elder, Greg, Barclay, Nicola, Wetherell, Mark and Ellis, Jason (2017) Anticipated next-day demand affects the magnitude of the cortisol awakening response, but not subjective or objective sleep. *Journal of Sleep Research*. ISSN 0962-1105 (In Press)

Published by: Wiley-Blackwell

URL: <https://doi.org/10.1111/jsr.12569> <<https://doi.org/10.1111/jsr.12569>>

This version was downloaded from Northumbria Research Link:
<http://nrl.northumbria.ac.uk/31152/>

Northumbria University has developed Northumbria Research Link (NRL) to enable users to access the University's research output. Copyright © and moral rights for items on NRL are retained by the individual author(s) and/or other copyright owners. Single copies of full items can be reproduced, displayed or performed, and given to third parties in any format or medium for personal research or study, educational, or not-for-profit purposes without prior permission or charge, provided the authors, title and full bibliographic details are given, as well as a hyperlink and/or URL to the original metadata page. The content must not be changed in any way. Full items must not be sold commercially in any format or medium without formal permission of the copyright holder. The full policy is available online: <http://nrl.northumbria.ac.uk/policies.html>

This document may differ from the final, published version of the research and has been made available online in accordance with publisher policies. To read and/or cite from the published version of the research, please visit the publisher's website (a subscription may be required.)

www.northumbria.ac.uk/nrl



Anticipated next-day demand affects the magnitude of the cortisol awakening response but not subjective or objective sleep

Running head: Anticipated demand affects the CAR but not sleep

Greg J. Elder^{*1}, Nicola L. Barclay², Mark A. Wetherell³, Jason G. Ellis³.

- 1) Institute of Neuroscience, Newcastle University, UK
- 2) Sleep and Circadian Neuroscience Institute, Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, UK
- 3) Northumbria Sleep Research Laboratory, Northumbria University, Newcastle upon Tyne, UK

*Corresponding Author

Dr. Greg J. Elder

Institute of Neuroscience, Newcastle University, Campus for Ageing and Vitality, Newcastle upon Tyne, UK, NE4 5PL, UK

Tel: +44 (0)191 208 1125

Email: greg.elder@ncl.ac.uk

Total number of words: 3,751

Number of references: 38

Conflict of interests: None

Author contributions: GE, NB, MW and JE conceived and conducted the study, interpreted the data and revised the manuscript. GE analysed and interpreted the data, and wrote the initial draft of the manuscript. All authors have read and approved the final version of the manuscript.

Summary

Whilst the association between sleep and stress is well-established, few studies have examined the effects of an anticipated stressor upon sleep and relevant physiological markers. The aim of the present study was to examine whether an anticipated stressor in the form of next-day demand affects subjective and objective sleep, and multiple indices of the cortisol awakening response (CAR). Subjective and objective sleep and the CAR were measured over three consecutive nights in 40 healthy adults in a sleep laboratory. During their second night, participants were informed that they would either be required to complete a series of demanding cognitive tasks, in a competition format, during the next day (anticipation condition; $n = 22$), or were given no instruction (sedentary condition; $n = 18$). Sleep was measured subjectively using sleep diaries, objectively using polysomnography, and saliva was measured at awakening, +15, +30, +45 and +60 minutes each morning, from which CAR measurement indices were derived: awakening cortisol levels, the mean increase in cortisol levels (MnInc) and total cortisol secretion. There were no between-group differences in subjective or objective sleep in the night preceding the anticipated demand, however, compared to the sedentary condition, those in the anticipation group displayed a larger MnInc, representing the CAR magnitude, on the morning of the anticipated demand. Overall, the results suggest that whilst anticipated stress affected the subsequent CAR, subjective and objective sleep remained undisturbed. It is possible that the timing of an anticipated stressor, rather than its expected duration, may influence subsequent sleep disruption.

Keywords: cortisol, stress, anticipation, polysomnography

Introduction

A range of studies have indicated that subjective and objective sleep disturbances are associated with stress (Bastien et al., 2004, Reynolds 3rd et al., 1992, Reynolds 3rd et al., 1993); that stressful events can predict future sleep disturbances (Lallukka et al., 2012, Vahtera et al., 2007); and that pre-sleep stress levels are predictive of subsequent subjective sleep quality (Åkerstedt et al., 2012). Whilst the link between stress and sleep is well-established, the impact of an anticipated stressor upon sleep is less clear, with the exception of a limited number of studies. In one such study, engineering staff completed sleep diaries whilst on board a ship, which included a twenty-four hour 'watch period' whereby they were allowed to sleep, but would be awakened by an automatic alarm system in the event of machinery malfunction (Torsvall et al., 1987). During the watch period, staff self-reported a reduced total sleep time and poorer sleep quality compared to free nights and interestingly, these disruptions were evident even when no alarms occurred. In a similar study, where sleep was measured objectively using polysomnography, a shorter duration of sleep and reduced amounts of REM and slow-wave sleep (SWS) were observed during watch periods, as compared to free nights (Torsvall and Akerstedt, 1988). Therefore, it could be concluded that even the anticipation of an upcoming stressor can affect subjective and objective sleep.

That said, two similar laboratory studies have investigated the effects of an anticipated stressor upon sleep, with very different outcomes. One study examined whether neuroticism, repression and coping style moderated the effects of an anticipated stressor upon REM sleep parameters (Germain et al., 2003). Healthy, good-sleepers were either allocated to a control group or a next-day stress condition, and were informed immediately before sleep that they were required to perform a speech, which would be evaluated, upon awakening. Interestingly, there were no group differences in terms of subjective sleep quality, or objective measures of sleep continuity or architecture. A second study from this group, using a similar anticipatory stress-induction paradigm, measured heart rate variability (HRV) as a marker of autonomic nervous system arousal (Hall et al., 2004). Whilst again there were no between-group differences in objective sleep continuity or architecture, more subtle differences were observed: participants in the stress condition demonstrated

lower parasympathetic modulation during NREM and REM sleep, and a higher sympathovagal balance during NREM sleep compared to the control group, representing an anticipatory stress response during the night. Taken together, the naturalistic studies, where the stressor may have occurred at any point over an eight-hour overnight period (Torsvall et al 1987; Torsvall & Akerstedt 1988) and stress-induction laboratory studies, which require action the subsequent morning, suggest that the timing of the anticipated stressor is potentially important.

Although Hall and colleagues measured HRV as a physiological marker of stress, to date, no studies have simultaneously examined the effects of anticipated stress upon sleep in tandem with a more robust, relevant and well-established physiological marker of stress. One such marker is cortisol, which is the end product of the hypothalamic-pituitary-adrenal (HPA) axis, an endocrine system which allows adjustment and adaptation to bodily and environmental demands (Fries et al., 2009). Cortisol is responsive to stress; acute psychological demand increases cortisol levels in a dose-response manner (Dickerson and Kemeny, 2004). One specific aspect of cortisol output which appears to be sensitive to an anticipated stressor is the cortisol awakening response (CAR), where cortisol levels sharply increase in response to morning awakening (Clow et al., 2004, Fries et al., 2009). Several ambulatory studies have indicated that the CAR is associated with periods of increased demand (Brant et al., 2010) or is a marker of anticipation of a forthcoming event (Baumler et al., 2014, Rohleder et al., 2007, Wetherell et al., 2015, Elder et al., 2016). For example, one study in preschool-aged children observed greater mean increases in cortisol levels between awakening and +30 minutes on the day of a prospective memory task (Baumler et al., 2014) and similarly, higher levels of cortisol have also been observed at +30 minutes on the day of a socially-evaluated laboratory event, when compared to control days (Wetherell et al., 2015). Therefore, the CAR is a potentially useful measure of the HPA axis response to an anticipated next-day stressor, particularly where the stressor is in the form of next-day demand.

The aim of the present study was to further elucidate the relationship between the anticipation of forthcoming stress, in terms of timing and duration, and sleep, in tandem with the CAR as a robust and sensitive physiological marker. Due to the exploratory nature of the study, there were

no specific hypotheses with regard to subjective and objective sleep, but it was expected that CAR indices would differ between individuals anticipating next-day demand and those expecting a sedentary day, where the CAR would be increased in those anticipating next-day demand.

Methods

Participants

A total of 40 healthy normal participants ($M_{\text{age}} = 23.44$ years, $SD_{\text{age}} = 3.40$ years) were recruited from the staff and student population of Northumbria University using email advertisements. Inclusion criteria required all participants to be healthy good sleepers. Exclusion criteria were current or previous sleep problems, physical illness, shift work, or trans-meridian travel in the three months prior to study enrolment. Participants provided written informed consent and the study was approved by Northumbria University Faculty of Health Sciences ethics committee. After consenting, participants were allocated to a sedentary ($n = 18$) or anticipation ($n = 22$) group. Both the anticipation and sedentary groups completed an identical baseline period, and slept in an identical sleep laboratory environment, where subjective sleep, objective sleep and the CAR were measured, and sleep and wake times were scheduled.

Procedure

The study procedure is summarised in Figure 1. Upon responding to the study invitation email, participants were provided with an information sheet and were invited to attend the sleep laboratory. Participants then provided informed consent and were confirmed as a healthy good sleeper by examining their sleep, psychiatric and physical illness history, and by completing the Pittsburgh Sleep Quality Index (PSQI; Buysse et al., 1989) and Hospital Anxiety and Depression Scale (HADS; Zigmond and Snaith, 1983).

The study consisted of a baseline period (Day -14 to Day 0) and a three-night weekday laboratory period (Night 0 - Day 3) as described previously (Elder et al., 2016). During the baseline period (Day -14 to Day 0), participants were instructed to complete self-report sleep diaries (Carney et

al., 2012) and to wear actigraphs in order to determine habitual sleep/wake schedules. Upon arrival at the sleep laboratory on Night 0, actigraphy data were visually inspected to confirm circadian stability. Sleep and wake times were scheduled in accordance with average baseline (Day -14 to Day 0) weekday sleep/wake times, derived from baseline sleep diaries.

Participants slept for three consecutive weekday nights in a sleep laboratory environment (Night 0 – Night 2) where sleep was measured objectively using polysomnography (PSG). The CAR was measured on each subsequent weekday morning (Day 1 - Day 3), where samples were collected by a researcher, in low-intensity ultraviolet light, between 0 and 60 minutes post-awakening, as described previously (Elder et al., 2016). Following the collection of saliva samples, participants completed subjective sleep diaries. Participants left the sleep laboratory approximately one hour after completion of the Day 1 sleep diary, and were instructed to continue with their habitual daily activities, before returning to the sleep laboratory on Night 1. Both groups of participants then remained in the sleep laboratory until Day 3 (though the experimental manipulation, described below, characterised their experience during this period), where saliva samples were collected as described. Participants were then debriefed and received a payment of £150 for their time.

Sedentary group

Participants in the sedentary group were informed that they would remain in the sleep laboratory on Day 2 and would be permitted to perform sedentary activities including reading, watching television or films. Participants were then allowed to sleep during Night 1 and saliva samples were collected on the morning of Day 2.

Anticipation group

Immediately before lights out on Night 1, participants in the anticipation group were provided with a standardised instruction sheet (Appendix A) informing them that they would remain in the sleep laboratory on Day 2, where they would be required to complete a range of demanding computerised cognitive tasks. In order to ensure that participants had a high level of motivation, participants in the anticipation group were also informed that the individual who attained the highest

score on a randomly-chosen task throughout the day would receive a high-value prize (Apple iPad). Participants were then allowed to sleep during Night 1, and saliva samples were subsequently collected on the morning of Day 2.

[Insert Figure 1]

Measures of sleep

Subjective sleep: Sleep diaries (Carney et al., 2012) were used to obtain measures of subjective sleep continuity (total sleep time (TST); time in bed, referring to the period of time between the participant going to bed and leaving bed the subsequent morning (TIB); sleep efficiency (SE%), calculated on the basis of $TST/TIB \times 100$; sleep-onset latency (SOL), referring to the length of time taken to get to sleep; the number of awakenings (NWAK), referring to the frequency of nocturnal awakenings during sleep; and wake after sleep onset (WASO), referring to the duration of time awake during the night after the initiation of sleep). Both groups also provided an indication of subjective stress, measured using a 100mm visual analogue scale (VAS) where the endpoints were anchored by “not at all” and “very much” in response to the statement “I feel stressed”.

Objective sleep: Polysomnography (PSG) was applied on each night and recording times were scheduled in accordance with average weekday sleep/wake times, derived from baseline (Day -14 to Day 0) sleep diaries. Electroencephalogram (EEG) electrodes were placed at FP₁, FP₂, F₃, F₄, C₃, C₄, P₃, P₄, O₁, O₂ and C_z, referenced to linked mastoids (M₁, M₂) and a ground electrode (FP_z). PSG also included electromyogram (EMG), electrooculogram (EOG) and electrocardiogram (ECG). PSG was obtained using a SOMNOscreen system (SOMNOmedics GmbH, Randersacker, Germany) and impedance levels were maintained below 5kΩ. Recordings were blind scored in 30-second epochs by an external scorer in accordance with American Academy of Sleep Medicine guidelines (Iber et al., 2007). From objective PSG data, measures of sleep continuity (total sleep time (TST); sleep efficiency (SE%); sleep onset latency (SOL); the number of awakenings (NWAK), wake after sleep

onset (WASO)) and sleep architecture (the percentages of wake, and percentages of sleep spent in rapid eye movement (REM), stage 1 (N1), stage 2 (N2) and stage 3 (N3) sleep; and the latency to each stage of sleep) were derived.

Cortisol awakening response

Saliva samples were obtained at awakening, +15, +30, +45 and +60 minutes using Salivettes (Sarstedt, Leicester, UK) and participants were required to chew on Salivettes for 60 seconds. Saliva samples were stored in a domestic refrigerator immediately following collection and were frozen at -20°C until assaying. Samples were centrifuged at 3000rpm for 15 minutes and all assays were performed in-house using the luminescence immunoassay method in line with manufacturer instructions (Salimetrics, Newmarket, UK; inter-assay coefficients of variability <10%).

Data Analysis

Alterations to objective sleep are typically observed during the first night in a sleep laboratory environment (known as the ‘first-night effect’; Agnew et al., 1966, Toussaint et al., 1995) and disturbed sleep may also affect the subsequent CAR. Therefore, in line with a previously-published protocol used to measure the CAR in a sleep laboratory environment (Elder et al., 2016), sleep data from the adaptation night (Night 0) and subsequent CAR data (Day 1) were excluded from analysis. As the aim of the study was to examine the effects of anticipated next-day demand upon sleep and the CAR, subjective and objective sleep data from Night 1, and CAR data from Day 2, are reported.

In order to examine the effects of anticipated demand upon subjective and objective sleep, Night 1 subjective measures of sleep continuity (TIB, TST, SE%, SOL, NWAK and WASO) and Night 1 objective measures of sleep continuity (TST, SE%, SOL, NWAK) and sleep architecture (percentages of sleep spent in REM, N1, N2, N3 and the latency to each stage of sleep) were compared between sedentary and demand groups using *t*-tests, where *p*-values were adjusted for multiple comparisons (subjective sleep continuity variables: adjusted *p*-value = 0.008 (0.05/6); objective sleep continuity variables: adjusted *p*-value = 0.013 (0.05/4); objective sleep architecture variables: adjusted *p*-value = 0.006 (0.05/8)).

CAR data from a total of five participants (sedentary $n = 3$; anticipation $n = 2$) were excluded due to saliva samples containing an insufficient volume of saliva for further analyses ($n = 4$), and due to excessively high (>75 nmol/l) (Kunz-Ebrecht et al., 2004) cortisol levels ($n = 1$). To examine the effects of next-day demand upon the CAR, Day 2 cortisol levels (expressed in nanomoles per litre (nmol/l), derived from saliva samples) were compared between sedentary and demand groups at each measurement. The CAR was further examined by comparing three additional Day 2 CAR measurement indices between sedentary and demand groups using t -tests: awakening cortisol levels, the mean increase in cortisol levels during the measurement period (MnInc; calculated using the average cortisol levels from all post-awakening samples (Wüst et al., 2000)) and total cortisol secretion, calculated on the basis of the area under the curve with respect to ground formula (AUC_G), expressed in arbitrary units. **These additional** CAR measurement indices were adjusted for multiple comparisons (adjusted p -value = 0.017 (0.05/3). **Effect sizes are reported using Cohen's d .**

Results

A total of 40 participants provided complete Night 1 subjective and objective sleep data ($M_{age} = 23.44$ years, $SD_{age} = 3.40$ years; sedentary $n = 18$, anticipation $n = 22$) and 35 participants provided complete CAR data ($M_{age} = 23.57$ years, $SD_{age} = 3.60$ years; sedentary $n = 15$, anticipation $n = 20$). There were no significant differences between sedentary and anticipation groups in terms of age, gender, PSQI or HADS scores (p -values > 0.05 ; Table 1). Additionally, there were no significant differences between sedentary and anticipation groups in terms of subjective measures of sleep continuity derived from baseline sleep diaries (all p -values > 0.008 ; Table 2).

[Insert Table 1]

[Insert Table 2]

Effects of anticipated next-day demand upon Night 1 sleep

There were no significant differences in subjective or objective measures of sleep, or subjective stress, between sedentary and anticipation groups during Night 1 (all p -values > 0.05 ; Tables 3 and 4).

[Insert Table 3]

[Insert Table 4]

Effects of anticipated next-day demand upon the cortisol awakening response

There was a significant main effect of time point upon cortisol levels (Figure 3), reflecting the typical increase in cortisol levels during the CAR measurement period ($F(2.99, 98.68) = 7.28, p < 0.001, \eta^2_p = 0.18$). The main effect of group was not significant ($F(1,33) = 0.99, p = 0.326, \eta^2_p = 0.03$). The time point \times group interaction was significant ($F(2.99,98.68) = 3.52, p = 0.018, \eta^2_p = 0.10$); however, *post-hoc* comparisons of cortisol levels between groups at each sampling point were not significant (all p -values > 0.05).

Comparisons of additional CAR indices showed that participants in the anticipation group displayed a significantly greater Day 2 increase in cortisol levels (MnInc) than sedentary participants ($t(33) = -2.66, p = 0.016$). There were no significant differences between sedentary and demand groups in terms of Day 2 awakening cortisol levels ($t(33) = 0.63, p = 0.309$) or total cortisol secretion (AUC_G) ($t(33) = -1.04, p = 0.315$). This is summarised in Table 5. Of note, results remained consistent with those reported when controlling for sex, menstrual cycle phase and oral contraceptive use (results unreported but available upon request from the first author).

[Insert Figure 3]

[Insert Table 5]

Discussion

The aim of this study was to examine the effects of an anticipated stressor, in the form of anticipated next-day demand, upon subjective sleep, objective sleep and the CAR. No differences in either subjective or objective sleep were observed between those anticipating demand and those anticipating a sedentary day the night prior. The anticipation of next-day demand did however, influence the CAR: a larger mean increase in cortisol levels was observed, representing the magnitude of the CAR, in those anticipating next-day demand compared to those anticipating a sedentary day. These results therefore support the proposed role of the CAR as an anticipatory process (Clow et al., 2010, Fries et al., 2009).

There were no between-group differences in subjective sleep, or objective measures of sleep continuity or architecture. These results are in line with previous stress-induction studies, where the anticipated stressor was expected to occur on the subsequent morning (Germain et al., 2003, Hall et al., 2004). However, in the present study, whilst the timing of the stressor was matched to previous laboratory stress-induction studies (Hall et al., 2004, Germain et al., 2003), the expected duration of the stressor was matched to previous naturalistic studies (Torsvall and Akerstedt, 1988, Torsvall et al., 1987). It is possible that laboratory stress-induction paradigms do not affect sleep if the stressor is **subjectively mild in nature**, not personally relevant, **or alternatively, not emotionally relevant**. **Indeed, there were no group differences in subjectively-rated stress**. That said, our participants in the anticipation group were aware that a high-value prize would be conferred to the individual attaining the highest score on a computerised task during the testing day. This unpredictability of the outcome of the stressor is akin to previous naturalistic studies, but was perhaps not meaningful enough to participants to disrupt sleep.

Although an anticipatory response was observed in terms of an increased CAR magnitude, the present study potentially indicates that the timing of the anticipated stressor is important, and an alternative explanation for the lack of sleep disruption is that night-time sleep may only be affected when the stressor is expected to occur during the night. This is indicated by previous naturalistic studies (Torsvall and Akerstedt, 1988, Torsvall et al., 1987), and also by a recent laboratory-based

study which simulated an ‘on-call’ situation (Wuyts et al., 2012): participants anticipated a task which required a response, however, the task did not occur. In this study, compared to a reference night without the anticipated stressor, participants displayed a trend towards reduced subjective total sleep time, reduced subjective sleep efficiency and increased percentage of subjective wake after sleep onset following the experimental night. Overall, this would indicate that it is the expected timing, and not the duration, of an anticipated stressor which is important with regards to subsequent sleep disruption.

A particular strength of the present study is in the measurement of the CAR in a highly-controlled laboratory environment, which compared to ambulatory studies, ensured that there was a complete level of participant adherence to the saliva sampling protocol. In the current study, saliva samples were obtained every 15 minutes during the CAR period with strict levels of monitoring. This is important since even short delays in the collection of saliva samples during the CAR period can produce erroneous results, and where the accurate collection of the awakening sample is vitally important (Smyth et al., 2013, Thorn et al., 2006, Griefahn and Robens, 2010). Whilst ambulatory studies can attempt to maximise participant adherence through the use of methods such as electronic time-stamped saliva collection tubes (Kudielka et al., 2007), in ambulatory studies where samples are collected every 15 minutes, participants can potentially become over-burdened and not adhere to collection instructions (Wetherell and Montgomery, 2014).

In the present study, there were no differences between sedentary and demand groups in terms of objective sleep continuity or architecture; these are factors which have the potential to influence subsequent CAR indices and in particular the CAR magnitude. In one sleep laboratory study, a positive association between the percentage of N2 sleep and the MnInc of the subsequent CAR was observed (Elder et al., 2016). In a separate study, positive associations were observed between the percentage of time spent in N2 sleep and the maximum increase in cortisol levels, and between the percentage of time spent in N1 sleep and awakening cortisol levels (Devine and Wolf, 2016). The increased CAR magnitude shown by the demand group is therefore likely to be caused by the

anticipated demand and not influenced by between-group differences in sleep continuity or architecture.

The main limitation of this study is in the limited sample size, which is primarily due to the intensive nature of the study protocol. Whilst this may have affected the comparison of the CAR profile between the two groups, significant between-group differences in the CAR magnitude were still observed. However, this limitation must be considered in light of the fact that the sleep laboratory environment offered a significant level of control over factors known to influence the subsequent CAR; for example, in the present study, there were no intra-individual differences in light (Figueiro and Rea, 2012, Scheer and Buijs, 1999). This is extremely important since the suprachiasmatic nucleus, which controls the co-ordination of the HPA axis, is particularly sensitive to light (Buijs et al., 2003).

Although in the present study gross EEG, in terms of objective sleep continuity and sleep architecture, were unaffected by an anticipated next-day stressor, high-frequency EEG activity may still be affected, since an anticipated stressor in a naturalistic situation has previously been shown to result in a reduction in EEG power density during the first sleep cycle (Torsvall and Akerstedt, 1988), although it is not known whether this is also the case in a highly-controlled laboratory situation. Additionally, the present study only examined the effects of an anticipated stressor over the course of one day. Whilst the duration of the stressor in the present study was matched to that of naturalistic studies, which observed disruptions to subjective and objective sleep, the relatively short duration of the stressor may still have enabled participants to ‘bank’ additional resources (Ellis et al., 2012) and mitigate the effect of the stressor upon sleep. Whilst the stressor employed in the present study was of a longer duration compared to previous laboratory stress-induction studies, subjective and objective sleep continuity and architecture may be disrupted by an anticipated stressor which occurs over multiple days. Additionally, it is not known whether a laboratory stressor can affect subjective and objective sleep after the stress has occurred, where a ‘rebound’ effect is observed.

Overall, we conclude that anticipated stress affects the subsequent CAR magnitude, but does not affect either subjective or objective sleep. Sleep disruption may be influenced by the subjective

perception of stress, personal salience of the stressor, or the timing of an anticipated stressor rather than its expected duration.

Acknowledgments

We would like to thank all study participants and Anthea Wilde for conducting the cortisol assays. We would also like to thank Dr. Zoe Gotts, Dr. Rachel Sharman and Dr. Umair Akram for their assistance with data collection. This study was financially supported by Northumbria University.

References

- Agnew, H. W., Webb, W. B. and Williams, R. L. The first night effect: An EEG study of sleep. *Psychophysiology*, 1966, 2: 263-66.
- Åkerstedt, T., Orsini, N., Petersen, H., Axelsson, J., Lekander, M. and Kecklund, G. Predicting sleep quality from stress and prior sleep – A study of day-to-day covariation across six weeks. *Sleep Med*, 2012, 13: 674-79.
- Bastien, C. H., Vallieres, A. and Morin, C. M. Precipitating factors of insomnia. *Behav. Sleep. Med.*, 2004, 2: 50-62.
- Baumler, D., Kliegel, M., Kirschbaum, C., Miller, R., Alexander, N. and Stalder, T. Effect of a naturalistic prospective memory-related task on the cortisol awakening response in young children. *Biol. Psychol.*, 2014, 103: 24-26.
- Brant, H., Wetherell, M. A., Lightman, S., Crown, A. and Vedhara, K. An exploration into physiological and self-report measures of stress in pre-registration doctors at the beginning and end of a clinical rotation. *Stress*, 2010, 13: 155-62.
- Buijs, R. M., Van Eden, C. G., Goncharuk, V. D. and Kalsbeek, A. The biological clock tunes the organs of the body: timing by hormones and the autonomic nervous system. *J. Endocrinol.*, 2003, 177: 17-26.
- Buysse, D. J., Reynolds Iii, C. F., Monk, T. H., Berman, S. R. and Kupfer, D. J. The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. *Psychiatry Res.*, 1989, 28: 193-213.
- Carney, C. E., Buysse, D. J., Ancoli-Israel, S. *et al.* The consensus sleep diary: standardizing prospective sleep self-monitoring. *Sleep*, 2012, 35: 287-302.
- Clow, A., Hucklebridge, F., Stalder, T., Evans, P. and Thorn, L. The cortisol awakening response: more than a measure of HPA axis function. *Neurosci. Biobehav. Rev.*, 2010, 35: 97-103.

- Clow, A., Thorn, L., Evans, P. and Hucklebridge, F. The awakening cortisol response: methodological issues and significance. *Stress*, 2004, 7: 29-37.
- Devine, J. K. and Wolf, J. M. Determinants of cortisol awakening responses to naps and nighttime sleep. *Psychoneuroendocrinology*, 2016, 63: 128-34.
- Dickerson, S. S. and Kemeny, M. E. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychol. Bull.*, 2004, 130: 355-91.
- Elder, G. J., Ellis, J. G., Barclay, N. L. and Wetherell, M. A. Assessing the daily stability of the cortisol awakening response in a controlled environment. *BMC Psychology*, 2016, 4: 1-10.
- Ellis, J. G., Gehrman, P., Espie, C. A., Riemann, D. and Perlis, M. L. Acute insomnia: Current conceptualizations and future directions. *Sleep Med Rev*, 2012, 16: 5 - 14.
- Figueiro, M. G. and Rea, M. S. Short-wavelength light enhances cortisol awakening response in sleep-restricted adolescents. *Int J Endocrinol*, 2012, 301935: 1-7.
- Fries, E., Dettenborn, L. and Kirschbaum, C. The cortisol awakening response (CAR): facts and future directions. *Int. J. Psychophysiol.*, 2009, 72: 67-73.
- Germain, A., Buysse, D. J., Ombao, H., Kupfer, D. J. and Hall, M. Psychophysiological reactivity and coping styles influence the effects of acute stress exposure on rapid eye movement sleep. *Psychosom. Med.*, 2003, 65: 857-64.
- Griefahn, B. and Robens, S. The normalization of the cortisol awakening response and of the cortisol shift profile across consecutive night shifts--an experimental study. *Psychoneuroendocrinology*, 2010, 35: 1501-9.
- Hall, M., Vasko, R., Buysse, D. *et al.* Acute stress affects heart rate variability during sleep. *Psychosom. Med.*, 2004, 66: 56-62.

- Iber, C., Ancoli-Israel, S. and Quan, S. F. *The AASM manual for the scoring of sleep and associated events: rules, terminology and technical specifications*. American Academy of Sleep Medicine, Westchester, 2007.
- Kudielka, B. M., Hawkley, L. C., Adam, E. K. and Cacioppo, J. T. Compliance with ambulatory saliva sampling in the Chicago Health, Aging, and Social Relations Study and associations with social support. *Ann. Behav. Med.*, 2007, 34: 209-16.
- Kunz-Ebrecht, S. R., Kirschbaum, C., Marmot, M. and Steptoe, A. Differences in cortisol awakening response on work days and weekends in women and men from the Whitehall II cohort. *Psychoneuroendocrinology*, 2004, 29: 516-28.
- Lallukka, T., Ferrie, J. E., Kivimäki, M., Shipley, M. J., Rahkonen, O. and Lahelma, E. Economic difficulties and subsequent sleep problems: Evidence from British and Finnish occupational cohorts. *Sleep Med*, 2012, 13: 680-85.
- Reynolds 3rd, C. F., Hoch, C. C., Buysse, D. J. *et al.* Electroencephalographic sleep in spousal bereavement and bereavement-related depression of late life. *Biol. Psychiatry*, 1992, 31: 69-82.
- Reynolds 3rd, C. F., Hoch, C. C., Buysse, D. J. *et al.* Sleep after spousal bereavement: A study of recovery from stress. *Biol. Psychiatry*, 1993, 34: 791-97.
- Rohleder, N., Beulen, S. E., Chen, E., Wolf, J. M. and Kirschbaum, C. Stress on the dance floor: The cortisol stress response to social-evaluative threat in competitive ballroom dancers. *Pers. Soc. Psychol. Bull.*, 2007, 33: 69-84.
- Scheer, F. a. J. L. and Buijs, R. M. Light affects morning salivary cortisol in humans. *J. Clin. Endocrinol. Metab.*, 1999, 84: 3395-98.

- Smyth, N., Clow, A., Thorn, L., Hucklebridge, F. and Evans, P. Delays of 5–15 min between awakening and the start of saliva sampling matter in assessment of the cortisol awakening response. *Psychoneuroendocrinology*, 2013, 38: 1476-83.
- Thorn, L., Hucklebridge, F., Evans, P. and Clow, A. Suspected non-adherence and weekend versus week day differences in the awakening cortisol response. *Psychoneuroendocrinology*, 2006, 31: 1009-18.
- Torsvall, L. and Åkerstedt, T. Disturbed sleep while being on-call: an EEG study of ships' engineers. *Sleep*, 1988, 11: 35-38.
- Torsvall, L., Castenfors, K., Åkerstedt, T. and Fröberg, J. a. N. Sleep at sea: a diary study of the effects of unattended machinery space watch duty. *Ergonomics*, 1987, 30: 1335-40.
- Toussaint, M., Luthringer, R., Schaltenbrand, N. *et al.* First-night effect in normal subjects and psychiatric inpatients. *Sleep*, 1995, 18: 463-9.
- Vahtera, J., Kivimaki, M., Hublin, C. *et al.* Liability to anxiety and severe life events as predictors of new-onset sleep disturbances. *Sleep*, 2007, 30: 1537-46.
- Wetherell, M. A., Lovell, B. and Smith, M. A. The effects of an anticipated challenge on diurnal cortisol secretion. *Stress*, 2015, 18: 42-48.
- Wetherell, M. A. and Montgomery, C. Basal functioning of the hypothalamic-pituitary-adrenal (HPA) axis and psychological distress in recreational ecstasy polydrug users. *Psychopharmacology*, 2014, 231: 1365-75.
- Wüst, S., Wolf, J., Hellhammer, D. H., Federenko, I., Schommer, N. and Kirschbaum, C. The cortisol awakening response - normal values and confounds. *Noise & Health*, 2000, 2: 79-88.
- Wuyts, J., De Valck, E., Vandekerckhove, M. *et al.* Effects of pre-sleep simulated on-call instructions on subsequent sleep. *Biol. Psychol.*, 2012, 91: 383-88.

Zigmond, A. S. and Snaith, R. P. The Hospital Anxiety and Depression Scale. *Acta Psychiatr. Scand.*,
1983, 67: 361-70.

Figure 1: Participant Flow

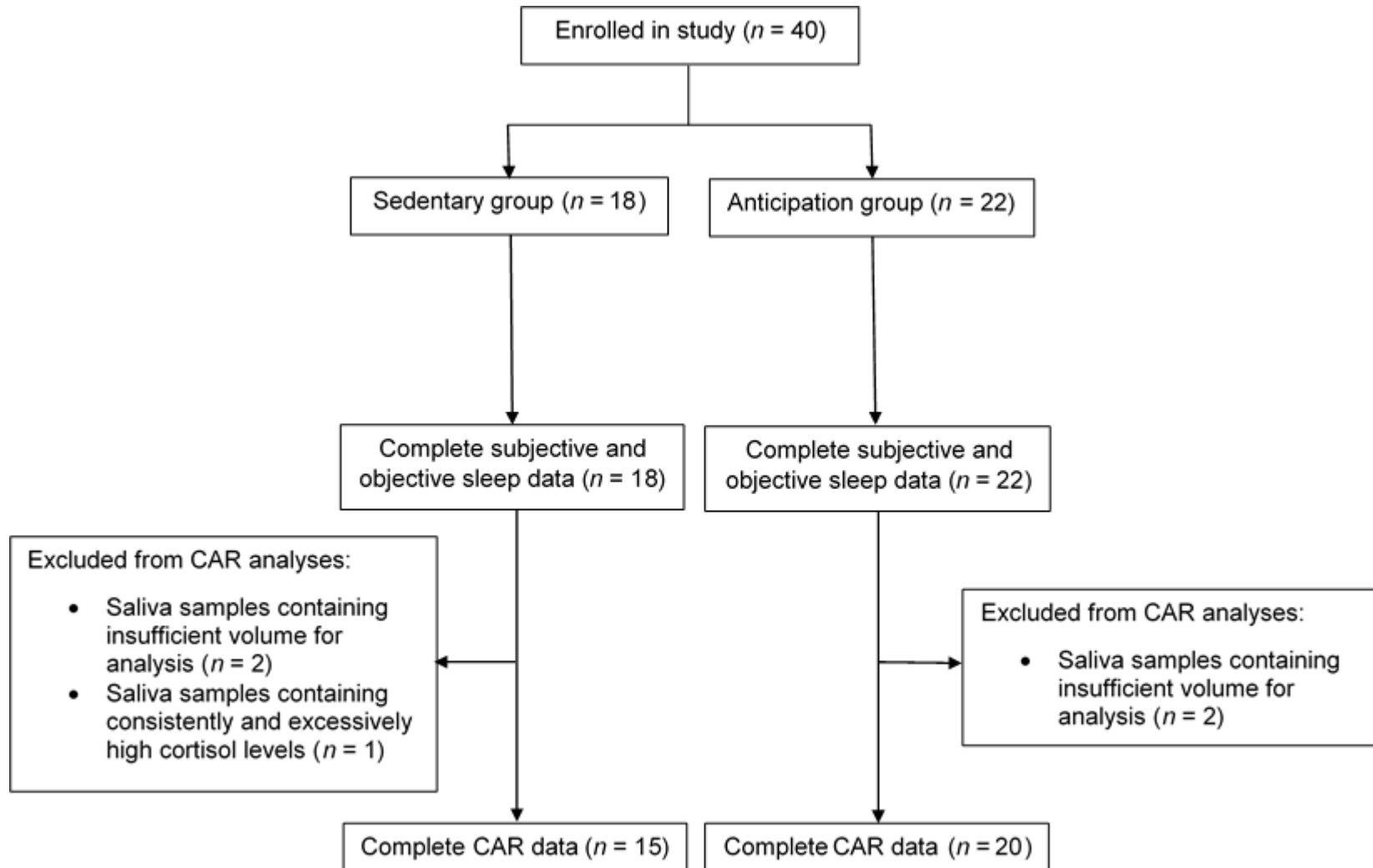
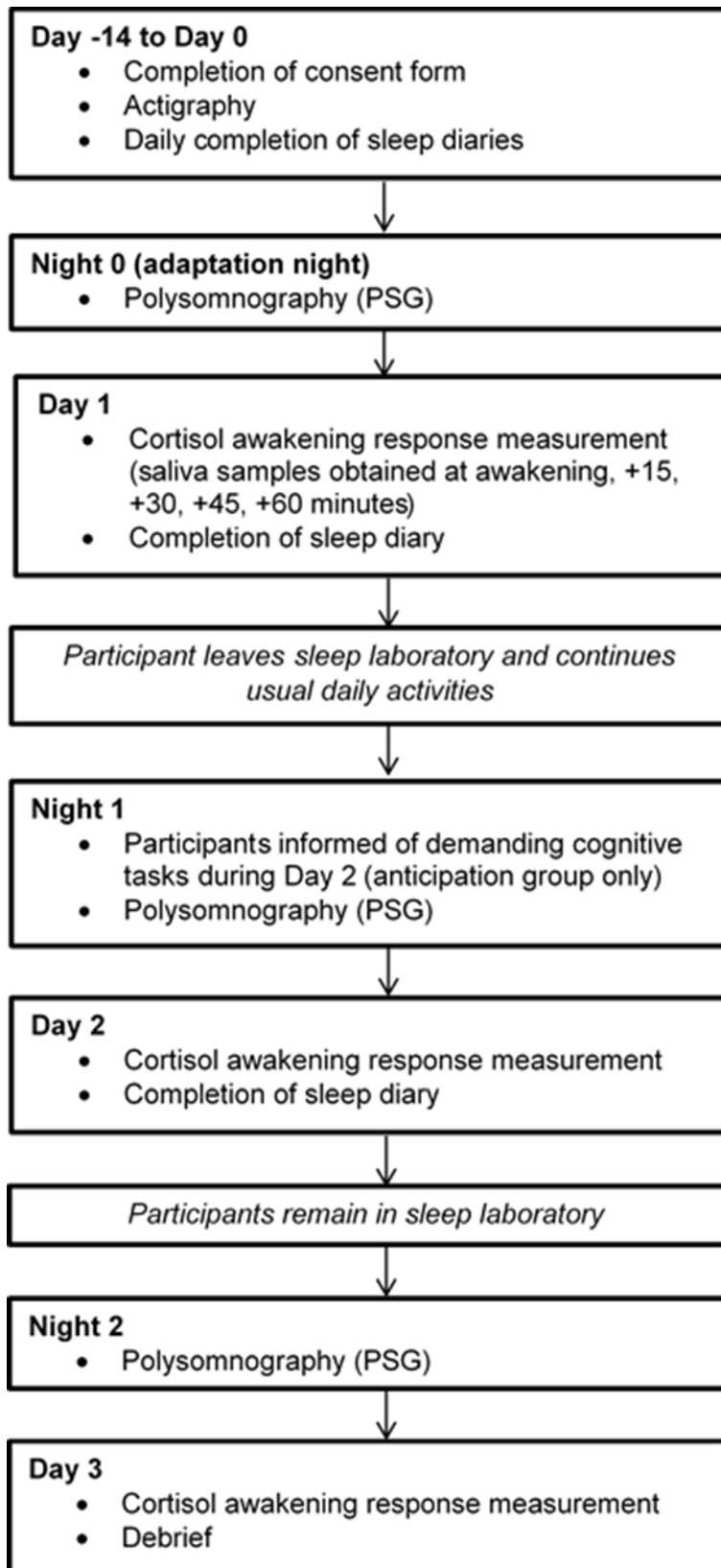


Figure 2: Study Schematic



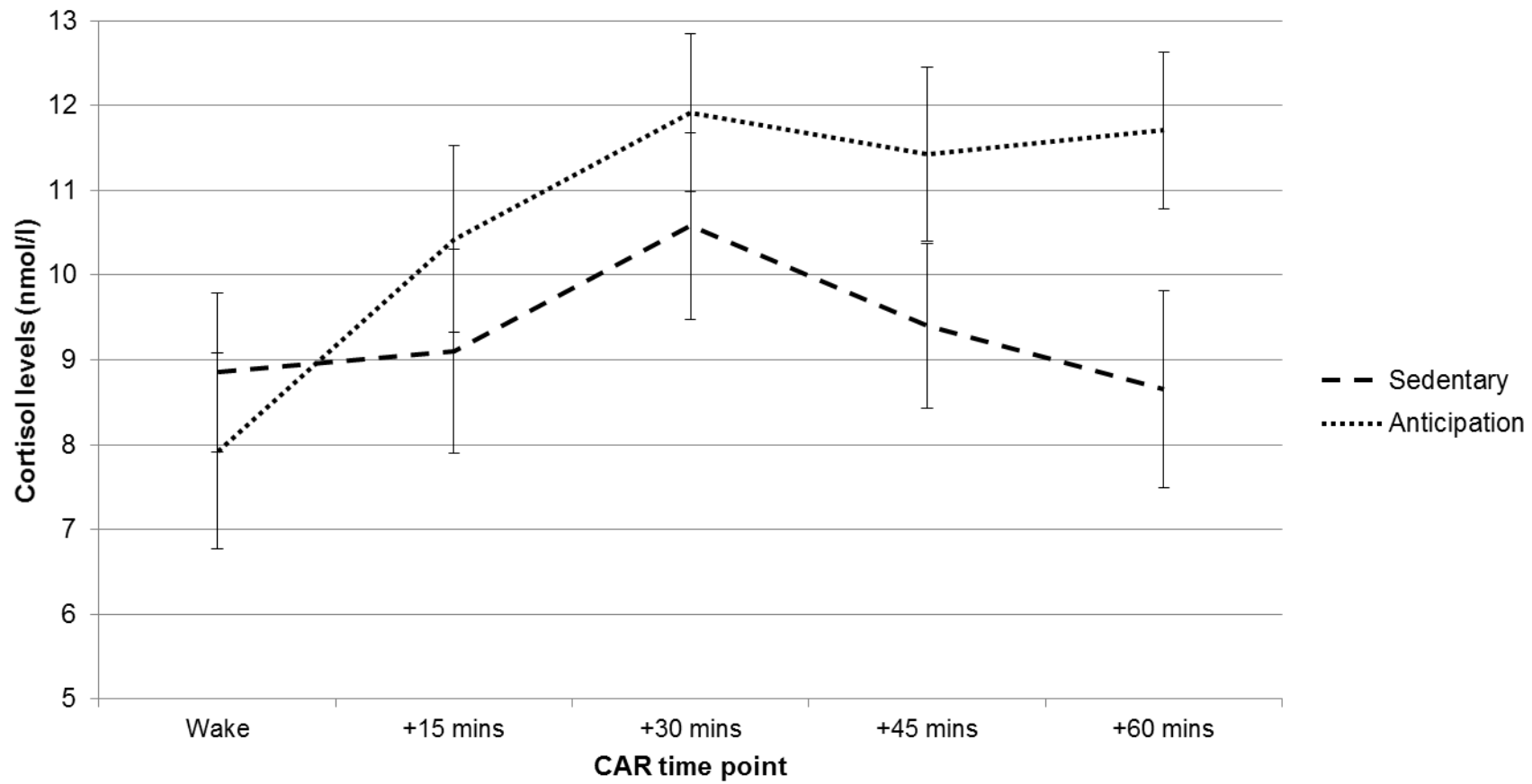


Figure 3: Day 2 mean (\pm SEM) cortisol levels at each CAR measurement point

Table 1:

Participant questionnaire comparisons

	Sedentary (<i>n</i> = 18)		Anticipation (<i>n</i> = 22)	
	Mean	<i>SD</i>	Mean	<i>SD</i>
Age (years)	23.46	3.21	23.42	3.62
Gender	9 male / 9 female		11 male / 11 female	
PSQI	3.56	1.34	3.36	1.89
HADS Anxiety	5.61	2.85	5.41	3.17
HADS Depression	2.00	1.97	2.36	1.97

Abbreviations: PSQI: Pittsburgh Sleep Quality Index; HADS: Hospital Anxiety and Depression Scale.

Table 2:

Baseline sleep diary subjective sleep continuity comparisons

	Sedentary (<i>n</i> = 18)		Anticipation (<i>n</i> = 22)		<i>p</i> -value	Effect size (<i>d</i>)
	Mean	<i>SD</i>	Mean	<i>SD</i>		
TIB (mins)	543.78	66.24	545.80	53.30	0.915	0.03
TST (mins)	472.25	50.12	476.70	58.25	0.800	0.08
SOL (mins)	16.67	7.73	16.86	13.52	0.956	0.02
NWAK	.99	.85	.84	.62	0.529	0.21
WASO (mins)	8.56	10.03	9.71	14.92	0.782	0.09
SE (%)	88.77	5.87	87.38	7.13	0.510	0.22

Abbreviations: TIB: time in bed, TST: total sleep time, SOL: sleep onset latency, NWAK: number of awakenings, WASO: wake after sleep onset, SE: sleep efficiency.

Table 3:

Night 1 subjective sleep continuity comparisons between sedentary and anticipation groups

	Sedentary (<i>n</i> = 18)		Anticipation (<i>n</i> = 22)		<i>p</i> -value	Effect size (<i>d</i>)
	Mean	SD	Mean	SD		
TIB (mins)	521.67	26.40	534.55	41.83	0.244	0.37
TST (mins)	448.89	27.20	457.64	55.99	0.548	0.20
SOL (mins)	15.00	6.64	19.43	18.14	0.332	0.32
NWAK	1.47	1.36	1.36	1.07	0.779	0.09
WASO (mins)	7.89	7.21	7.73	9.31	0.952	0.02
SE (%)	86.17	5.47	85.56	8.33	0.791	0.09
Subjective stress (mm)	26.94	18.09	22.33 ^a	17.39 ^a	0.423	0.27

Abbreviations: TIB: time in bed, TST: total sleep time, SOL: sleep onset latency, NWAK: number of awakenings, WASO: wake after sleep onset, SE: sleep efficiency.

^a(*n* = 21)

Table 4:

Night 1 anticipation and sedentary group objective sleep comparisons

	Sedentary (<i>n</i> = 18)		Anticipation (<i>n</i> = 22)		<i>p</i> -value	Effect size (<i>d</i>)
	Mean	<i>SD</i>	Mean	<i>SD</i>		
TST (mins)	434.78	23.44	452.80	41.21	0.092	0.54
SOL (mins)	12.28	9.47	10.25	7.21	0.447	0.25
NWAK	13.78	5.78	12.09	4.95	0.326	0.32
WASO (mins)	12.08	7.27	8.68	4.31	0.074	0.60
SE (%)	94.71	2.19	95.96	1.89	0.060	0.63
Time in REM (%)	22.59	4.17	24.07	6.15	0.390	0.28
Time in N1 (%)	3.38	1.41	3.30	1.85	0.883	0.05
Time in N2 (%)	54.45	5.76	51.06	8.33	0.152	0.48
Time in N3 (%)	19.58	5.12	21.58	5.86	0.263	0.37
Latency to REM (mins)	109.75	39.92	95.80	36.67	0.257	0.38
Latency to N1 (mins)	12.28	9.47	10.25	7.21	0.447	0.25
Latency to N2 (mins)	17.19	11.08	16.41	12.42	0.836	0.07
Latency to N3 (mins)	29.50	12.63	27.68	12.82	0.656	0.15

Abbreviations: TST: total sleep time, SOL: sleep onset latency, NWAK: number of awakenings, WASO: wake after sleep onset, SE: sleep efficiency, REM: rapid eye movement sleep, N1: stage 1 sleep, N2: stage 2 sleep, N3: stage 3 sleep.

Table 5:

Day 2 additional cortisol awakening response measurement indices by group

	Sedentary ($n = 15$)		Anticipation ($n = 20$)		<i>p</i> -value	Effect size (<i>d</i>)
	Mean	<i>SD</i>	Mean	<i>SD</i>		
Awakening levels (nmol/l)	8.85	4.47	7.92	4.18	0.309	0.22
MnInc (nmol/l)	.58*	2.58	3.45*	3.51	0.016	0.94
AUC _G (arbitrary units)	567.50	219.04	653.62	258.60	0.315	0.37

Abbreviations: AUC_G : area under the curve with respect to ground, MnInc: mean increase.

* $p < .017$

Appendix A

Night 1 task written instructions (anticipation group)

“On your second day in the sleep laboratory, you will be required to complete a range of mentally demanding computerised tests at regular intervals throughout the day. Your performance on each task will be carefully monitored and it is very important that you perform as well as you possibly can on these tasks. Each task will take between 10 and 20 minutes to complete. These tasks will be completed from the moment you wake until you go to bed. We will monitor the amount of effort that you are putting into each task.

Your tests will include a measure of attention, where you have to press the coloured key on the computer keyboard corresponding with the letter of the word you are shown. It is important that you do this as quickly as you possibly can. You will also have to complete a combination of four tasks where you have to perform several tasks at once. You will have to complete this as quickly and accurately as possible, and you will also need to try to achieve the highest score you possibly can. In this task you will be able to see your score, and you should do your very best to beat your score each time as you will complete this particular task at various points during the day. It is very important that you make sure that you are performing all of the tasks to the best of your ability. The final task which you will complete is a decision-making card task, and again your job is to work out what the rules are, and to achieve as high a score as you possibly can. You will have the chance to win an iPad if you achieve the highest score on a randomly-selected task.”